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**(54) Title:** PLANT PATHOGEN RESISTANCE GENES AND USES THEREOF**(57) Abstract**

The *Arabidopsis RPP5* gene has been cloned and its sequence provided, along with the encoded amino acid sequence. DNA encoding the polypeptide, and alleles, mutants and derivatives thereof, may be introduced into plant cells and the encoded polypeptide expressed, conferring pathogen resistance on plants comprising such cells and descendants thereof. The *RPP5* sequence comprises leucine rich repeats and the presence of such repeats enables identification of other plant pathogen resistance genes. Homologies between *RPP5* and other pathogen resistance genes reveal motifs useful in identification of other pathogen resistance genes.

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## PLANT PATHOGEN RESISTANCE GENES AND USES THEREOF

The present invention relates to pathogen resistance in plants and more particularly to the identification and use of pathogen resistance genes.

5 It is based on cloning of the *Arabidopsis RPP5* gene.

Plants are constantly challenged by potentially pathogenic microorganisms. Crop plants are particularly vulnerable, because they are usually grown as genetically uniform monocultures; when  
10 disease strikes, losses can be severe. However, most plants are resistant to most plant pathogens. To defend themselves, plants have evolved an array of both preexisting and inducible defences. Pathogens must specialize to circumvent the defence mechanisms  
15 of the host, especially those biotrophic pathogens that derive their nutrition from an intimate association with living plant cells. If the pathogen can cause disease, the interaction is said to be compatible, but if the plant is resistant, the  
20 interaction is said to be incompatible. Race specific resistance is strongly correlated with the hypersensitive response (HR), an induced response by which (it is hypothesized) the plant deprives the pathogen of living host cells by localized cell death  
25 at sites of attempted pathogen ingress.

It has long been known that HR-associated disease resistance is often (though not exclusively) specified by dominant genes (*R* genes). Flor showed that when

pathogens mutate to overcome such *R* genes, these mutations are recessive. Flor concluded that for *R* genes to function, there must also be corresponding genes in the pathogen, denoted avirulence genes (*Avr* genes). To become virulent, pathogens must thus stop making a product that activates *R* gene-dependent defence mechanisms (Flor, 1971). A broadly accepted working hypothesis, often termed the elicitor/receptor model, is that *R* genes encode products that enable plants to detect the presence of pathogens, provided said pathogens carry the corresponding *Avr* gene (Gabriel and Rolfe, 1990). This recognition is then transduced into the activation of a defence response.

Some interactions exhibit different genetic properties. *Helminthosporium carbonum* races that express a toxin (*Hc* toxin) infect maize lines that lack the *Hm1* resistance gene. Mutations to loss of *Hc* toxin expression are recessive, and correlated with loss of virulence, in contrast to gene-for-gene interactions in which mutations to virulence are recessive. A major accomplishment was reported in 1992, with the isolation by tagging of the *Hm1* gene (Johal and Briggs, 1992). Plausible arguments have been made for how gene-for-gene interactions could evolve from toxin-dependent virulence. For example, plant genes whose products were the target of the toxin might mutate to confer even greater sensitivity

to the toxin, leading to HR, and the conversion of a sensitivity gene to a resistance gene. However, this does not seem to be the mode of action of *Hml*, whose gene product inactivates Hc toxin.

5           Pathogen avirulence genes are still poorly understood. Several bacterial Avr genes encode hydrophilic proteins with no homology to other classes of protein, while others carry repeating units whose number can be modified to change the range of plants  
10           on which they exhibit avirulence (Keen, 1992; Long and Staskawicz, 1993). Additional bacterial genes (*hrp* genes) are required for bacterial Avr genes to induce HR, and also for pathogenicity (Keen, 1992; Long and Staskawicz, 1993). It is not clear why pathogens make  
15           products that enable the plant to detect them. It is widely believed that certain easily discarded Avr genes contribute to but are not required for pathogenicity, whereas other Avr genes are less dispensable (Keen, 1992; Long, et al, 1993). The  
20           characterization of two fungal avirulence genes has also been reported. The Avr9 gene of *Cladosporium fulvum*, which confers avirulence on *C. fulvum* races that attempt to attack tomato varieties that carry the *Cf-9* gene, encodes a secreted cysteine-rich peptide  
25           with a final processed size of 28 amino acids but its role in compatible interactions is not clear (De Wit, 1992). The Avr4 gene of *C. fulvum* encodes a secreted peptide that is processed to a final size of amino

acids 106 (Joosten et al, 1994)

The technology for gene isolation based primarily on genetic criteria has improved dramatically in recent years, and many workers are currently  
5 attempting to clone a variety of *R* genes.

The map based cloning of the tomato *Pto* gene that confers "gene-for-gene" resistance to the bacterial speck pathogen *Pseudomonas syringae* pv tomato (*Pst*) has been reported (Martin et al, 1993). A YAC (yeast  
10 artificial chromosome) clone was identified that carried restriction fragment length polymorphism (RFLP) markers that were very tightly linked to the gene. This YAC was used to isolate homologous cDNA clones. Two of these cDNAs were fused to a strong  
15 promoter, and after transformation of a disease sensitive tomato variety, one of these gene fusions was shown to confer resistance to *Pst* strains that carry the corresponding avirulence gene, *AvrPto*. These two cDNAs show homology to each other. Indeed, the *Pto*  
20 cDNA probe reveals a small gene family of at least six members, 5 of which can be found on the YAC from which *Pto* was isolated, and which thus comprise exactly the kind of local multigene family inferred from genetic analysis of other *R* gene loci.

25 The *Pto* gene cDNA sequence is puzzling for proponents of the simple elicitor/receptor model. It reveals unambiguous homology to serine/threonine kinases, consistent with a role in signal

transduction. Intriguingly, there is strong homology to the kinases associated with self incompatibility in Brassicas, which carry out an analogous role, in that they are required to prevent the growth of

5 genotypically defined incompatible pollen tubes. However, in contrast to the Brassica SRK kinase (Stein et al, 1991), the *Pto* gene appears to code for little more than the kinase catalytic domain and a potential N-terminal myristoylation site that could promote

10 association with membranes. It would be surprising if such a gene product could act alone to accomplish the specific recognition required to initiate the defence response only when the *AvrPto* gene is detected in invading microorganisms. The race-specific elicitor

15 molecule made by *Pst* strains that carry *AvrPto* is still unknown and needs to be characterized before possible recognition of this molecule by the *Pto* gene product can be investigated.

Since the isolation of the *Pto* gene a number of

20 other resistance genes have been isolated. The isolation of the tobacco mosaic resistance gene *N* from tobacco was reported by Whitham et al (1994). The isolation of the flax rust resistance gene *L6* from flax was reported by Lawrence et al (1995). The

25 isolation of two *Arabidopsis thaliana* genes for resistance to *Pseudomonas syringae* has been reported. The isolation of *RPS2* was reported by Bent et al (1994) and by Mindrinos et al (1994) and the isolation

of *RPM1* was reported by Grant et al (1995). These genes probably encode cytoplasmic proteins that carry a nucleotide binding site (NBS) and a leucine-rich repeat (LRR). The ligands with which they interact are uncharacterised and it is not known what other plant proteins they interact with to accomplish the defence response. Our own laboratory has reported the isolation of the tomato *Cf-9* gene which confers resistance against the fungus *Cladosporium fulvum*. This is disclosed in WO95/18230 and has been reported in Jones et al (1994). We have also cloned the tomato *Cf-2* gene, which confers resistance against *Cladosporium fulvum*; this is disclosed in an International patent application filed by us on 1 April 1996 claiming priority from GB 9506658.5 filed 31 March 1995 and has been reported in Dixon et al. (1996). Its structure resembles the *Cf-9* gene in that the DNA sequence predicts a protein which is predominantly extracellular, with many leucine-rich repeats and which carries a C-terminal putative membrane anchor. The *Xa21* gene of rice has also been cloned recently (Song et al., 1995). The predicted protein product of this gene exhibits an N-terminal, presumably extracellular, domain composed principally of leucine rich repeats similar to those of *Cf-9* and *Cf-2*, a predicted transmembrane domain, and a presumably cytoplasmic domain with strong similarities to serine-threonine protein kinases, particularly



that encoded by *Pto*.

The subject-matter of the present invention relates to "pathogen resistance genes" or "disease resistance genes" and uses thereof. A pathogen resistance gene (R) enables a plant to detect the presence of a pathogen expressing a corresponding avirulence gene (Avr). When the pathogen is detected, a defence response such as the hypersensitive response (HR) is activated. By such means a plant may deprive the pathogen of living cells by localised cell death at sites of attempted pathogen ingress. Other genes, including the PGIP gene of WO93/11241 (for example), are induced in the plant defence response resulting from detection of a pathogen by an R gene.

A pathogen resistance gene may be envisaged as encoding a receptor to a pathogen-derived and Avr dependent molecule. In this way it may be likened to the RADAR of a plant for detection of a pathogen. Genes involved in the defence the plant mounts to the pathogen once detected are not pathogen resistance genes. Expression of a pathogen resistance gene in a plant causes activation of a defence response in the plant. This may be upon contact of the plant with a pathogen or a corresponding elicitor molecule, though the possibility of causing activation by over-expression of the resistance gene in the absence of elicitor has been reported. The defence response may be activated locally, e.g. at a site of contact of the

plant with pathogen or elicitor molecule, or systemically. Activation of a defence response in a plant expressing a pathogen resistance gene may be caused upon contact of the plant with an appropriate, corresponding elicitor molecule. The elicitor may be contained in an extract of a pathogen such as *Peronospora parasitica*, or may be wholly or partially purified and may be wholly or partially synthetic. An elicitor molecule may be said to "correspond" if it is a suitable ligand for the R gene product to elicit activation of a defence response.

We have now isolated the *Arabidopsis RPP5* gene which confers resistance against the downy mildew fungus (*Peronospora parasitica*). We have sequenced the DNA and deduced the most likely amino acid sequence from this gene. The DNA sequence of the *Arabidopsis RPP5* genomic gene is shown in Figure 1 (SEQ ID NO. 1) and the deduced amino acid sequence is shown in Figure 2 (SEQ ID NO. 2). The prediction of the Amino acid sequence is based on the identification of introns by reverse transcriptase polymerase chain reaction using primers designed to the determined genomic sequence. The part of the DNA sequence that is presumed to be spliced into exons and encoding the *RPP5* polypeptide is shown in capital letters in Figure 1. Figure 4 (SEQ ID NO 5) shows a contiguous nucleotide sequence coding for the amino acid sequence of Figure 2, made by joining together the exons of the

sequence of Figure 1.

As described in more detail below, the *Arabidopsis RPP5* gene was isolated by map-based cloning. In this technique the locus that confers resistance is mapped at high resolution relative to restriction fragment length polymorphism (RFLP) markers that are linked to the resistance gene. We identified a marker that appeared to be absolutely linked to the resistance gene and used probes corresponding to this marker to isolate binary vector cosmid clones from a library made with DNA of an *Arabidopsis* landrace *Landsberg erecta* that carried the *RPP5* gene. A binary vector cosmid clone designated 29L17, on transformation into disease sensitive *Arabidopsis*, conferred disease resistance. DNA sequence analysis of the cloned DNA identified a gene with leucine-rich repeats. A subclone of 29L17, designated pRPP5-1, containing 6304 bp of DNA including 1298 bp 5' to the probable initiation codon (Figure 1) and 458 bp 3' to the probable termination codon was constructed in a binary vector. The subclone was used to transform *Arabidopsis* ecotype Columbia and shown to confer disease resistance. Analysis of a fast neutron induced mutation of *Landsberg* that had become disease sensitive revealed rearrangement of the DNA structure of this gene. Taken together these data provide the necessary evidence that the sequences as shown in Figures 1 and 2 correspond to the *RPP5* gene.

According to one aspect, the present invention provides a nucleic acid isolate encoding a pathogen resistance gene, the gene being characterized in that it encodes the amino acid sequence shown in SEQ ID NO 2, or a fragment thereof, or an amino acid sequence showing a significant degree of homology thereto. N and L6 may be excluded.

For instance, embodiments of nucleic acid according to the invention, e.g. encoding a polypeptide comprising an amino acid sequence that is a mutant, derivative, allele or variant of the sequence shown in Figure 2 (as discussed further herein), may be distinguished from other pathogen resistance genes such as N, L6 by optionally having any one or more of the following features:

the encoded polypeptide has less than 30% homology with the amino acid sequence of the tobacco N protein, shown in Figure 3 and less than 25% homology with the amino acid sequence of the flax L6 protein, shown in Figure 3;

its expression does not activate said defence response upon contact of the plant with a molecule that is an elicitor of the tobacco N protein;

its expression does not activate said defence response upon contact of the plant with a molecule that is an elicitor of the flax L6 protein;

its expression does not when in a tobacco plant activate said defence response upon contact of the

tobacco plant with Tobacco Mosaic Virus;

its expression does not when in a flax plant  
activate said defence response upon contact of the  
flax plant with *Melampsora lini*;

5 its expression does not activate said defence  
response upon contact of the plant with a molecule  
that is an elicitor of the *Arabidopsis* RPS2 protein;

its expression does not activate said defence  
response upon contact of the plant with a molecule  
10 that is an elicitor of the *Arabidopsis* RPM1 protein;

its expression does not when in *Arabidopsis*  
*thaliana* activate said defence response upon contact  
of the plant with *Pseudomonas syringae*;

the encoded polypeptide shows less than 20%  
15 homology with the amino acid sequence of the tomato  
Cf-9 protein and less than 20% homology with the amino  
acid sequence of a tomato Cf-2 protein;

its expression does not activate said defence  
response upon contact of the plant with a molecule  
20 that is an elicitor of the tomato Cf-9 protein nor  
with a molecule that is an elicitor of the tomato Cf-2  
protein;

its expression does not when in a tomato plant  
activate said defence response upon contact of the  
25 tomato plant with *Cladosporium fulvum* expressing an  
Avr2 molecule nor *Cladosporium fulvum* expressing an  
Avr9 molecule;

the encoded polypeptide comprises a putative

nucleotide binding site;

the encoded polypeptide is a cytoplasmic protein;

the encoded polypeptide comprises a region having  
homology to the cytoplasmic domain of the *Drosophila*  
5 Toll protein.

Another way of distinguishing nucleic acid  
according to the present invention from other pathogen  
resistance genes such as N and L6 may be for the  
encoded polypeptide to comprise an N-terminal domain  
10 that has greater than 60% homology with the amino acid  
sequence of the N-terminal domain of RPP5 shown in  
Figure 2 (encoded by exon 1 of Figure 1), and/or  
comprise a nucleotide binding site domain that has  
greater than 40% homology with the amino acid sequence  
15 of the domain of RPP5 shown in Figure 2 encoded by  
exon 2 of Figure 1, and/or comprise a domain that has  
greater than 30% homology with the amino acid sequence  
of the domain of RPP5 shown in Figure 2 encoded by  
exon 3 of Figure 1, and/or comprise a domain that has  
20 greater than 30% homology with the amino acid sequence  
of the leucine-rich repeat (LRR) domain of RPP5 shown  
in Figure 2 encoded by exons 4, 5 and 6 of Figure 1.

Table 2 shows % amino acid identity between  
putative domains of RPP5 and N, and RPP5 and L6, as  
25 encoded by exons of the genomic sequences.

The nucleic acid may comprise a sequence of  
nucleotides encoding an amino acid sequence showing at  
least about 60% homology, preferably at least about

70% homology, at least about 80% homology, or more preferably at least about 90% or greater homology to the amino acid sequence shown in SEQ ID NO 2.

Generally, "% amino acid homology" is used to refer to  
5 % amino acid identity. High homology may be indicated by ability of complementary nucleic acid to hybridise under appropriate conditions, for instance conditions stringent enough to exclude hybridisation to sequences not encoding a pathogen resistance gene. Thus, the  
10 words allele, derivative or mutant may in context be used in respect of any sequence of nucleotides capable of hybridising with any of the nucleotide sequences encoding a polypeptide comprising the relevant sequence of amino acids.

15 Most preferably the nucleic acid encodes the amino acid sequence shown in SEQ ID No 2 in which case the nucleic acid may comprise DNA with an encoding sequence shown in SEQ ID NO 1 or sufficient part to encode the desired polypeptide (eg from the initiating  
20 methionine codon to the first in frame downstream stop codon of the mRNA). In one embodiment, DNA comprises a sequence of nucleotides which are the nucleotides 1966 to 6511 of SEQ ID NO 1, or a mutant, derivative or allele thereof, for instance lacking introns.  
25 Figure 4 provides a contiguous sequence encoding the amino acid sequence of Figure 2.

A further aspect of the invention provides a nucleic acid isolate encoding a pathogen resistance

gene, or a fragment thereof, obtainable by screening a nucleic acid library with a probe comprising nucleotides 1966 to 6511 of SEQ ID NO 1, nucleotides complementary thereto, or a fragment, derivative, mutant or allele thereof, and isolating nucleic acid which encodes a polypeptide able to confer pathogen resistance to a plant. Suitable techniques are well known in the art. Thus, the present invention also provides a method of identifying and/or isolating nucleic acid encoding a pathogen resistance gene comprising probing candidate (or "target") nucleic acid with nucleic acid which has a sequence of nucleotides which encodes the amino acid sequence shown in Figure 2, which is complementary to an encoding sequence or which encodes a fragment of either an encoding sequence or a sequence complementary to an encoding sequence. The candidate nucleic acid (which may be, for instance, cDNA or genomic DNA) may be derived from any cell or organism which may contain or is suspected of containing nucleic acid encoding a pathogen resistance gene. A preferred nucleotide sequence appears in Figure 1. Sequences complementary to the sequence shown, and fragments thereof, may be used.

Preferred conditions for probing are those which are stringent enough for there to be a simple pattern with a small number of hybridisations identified as positive which can be investigated further. It is



well known in the art to increase stringency of hybridisation gradually until only a few positive clones remain.

Nucleic acid according to the present invention  
5 may encode the amino acid sequence shown in SEQ ID NO 2 or a mutant, derivative or allele of the sequence provided. Preferred mutants, derivatives and alleles are those which retain a functional characteristic of the protein encoded by the wild-type gene, especially  
10 the ability to confer pathogen resistance. Changes to a sequence, to produce a mutant or derivative, may be by one or more of insertion, deletion or substitution of one or more nucleotides in the nucleic acid, leading to the insertion, deletion or substitution of  
15 one or more amino acids. Of course, changes to the nucleic acid which make no difference to the encoded amino acid sequence are included.

The nucleic acid may be DNA or RNA and may be synthetic, eg with optimised codon usage for  
20 expression in a host organism of choice. Nucleic acid molecules and vectors according to the present invention may be provided isolated and/or purified from their natural environment, in substantially pure or homogeneous form, or free or substantially free of  
25 nucleic acid or genes of the species of interest or origin other than the sequence encoding a polypeptide with the required function. Nucleic acid according to the present invention may comprise cDNA, RNA, genomic

DNA and may be wholly or partially synthetic. The term "isolate" encompasses all these possibilities.

Also provided by an aspect of the present invention is nucleic acid comprising a sequence of  
5 nucleotides complementary to a nucleotide sequence hybridisable with any encoding sequence provided herein. Another way of looking at this would be for nucleic acid according to this aspect to be hybridisable with a nucleotide sequence complementary  
10 to any encoding sequence provided herein. Of course, DNA is generally double-stranded and blotting techniques such as Southern hybridisation are often performed following separation of the strands without a distinction being drawn between which of the strands  
15 is hybridising. Preferably the hybridisable nucleic acid or its complement encode a polypeptide able to confer pathogen resistance on a host, i.e. includes a pathogen resistance gene. Preferred conditions for hybridisation are familiar to those skilled in the  
20 art, but are generally stringent enough for there to be positive hybridisation between the sequences of interest to the exclusion of other sequences, i.e. sequences not encoding polypeptides able to confer pathogen resistance on a host.

25 The nucleic acid may be in the form of a recombinant vector, for example a phage or cosmid vector. The nucleic acid may be under the control of an appropriate promoter and regulatory elements for

expression in a host cell, for example a plant cell. In the case of genomic DNA, this may contain its own promoter and regulatory elements and in the case of cDNA this may be under the control of an appropriate promoter and regulatory elements for expression in the host cell.

Those skilled in the art are well able to construct vectors and design protocols for recombinant gene expression. Suitable vectors can be chosen or constructed, containing appropriate regulatory sequences, including promoter sequences, terminator fragments, polyadenylation sequences, enhancer sequences, marker genes and other sequences as appropriate. For further details see, for example, *Molecular Cloning: a Laboratory Manual*: 2nd edition, Sambrook et al, 1989, Cold Spring Harbor Laboratory Press. Many known techniques and protocols for manipulation of nucleic acid, for example in preparation of nucleic acid constructs, mutagenesis, sequencing, introduction of DNA into cells and gene expression, and analysis of proteins, are described in detail in *Short Protocols in Molecular Biology*, Second Edition, Ausubel et al. eds., John Wiley & Sons, 1992. The disclosures of Sambrook et al. and Ausubel et al. are incorporated herein by reference.

When introducing a chosen gene construct into a cell, certain considerations must be taken into account, well known to those skilled in the art. The

nucleic acid to be inserted may be assembled within a construct which contains effective regulatory elements which will drive transcription. There must be available a method of transporting the construct into  
5 the cell. Once the construct is within the cell membrane, integration into the endogenous chromosomal material may or may not occur according to different embodiments of the invention. Finally, as far as plants are concerned the target cell type must be such  
10 that cells can be regenerated into whole plants.

Plants transformed with a DNA segment containing pre-sequence may be produced by standard techniques which are already known for the genetic manipulation of plants. DNA can be transformed into plant cells  
15 using any suitable technology, such as a disarmed Ti-plasmid vector carried by Agrobacterium exploiting its natural gene transfer ability (EP-A-270355, EP-A-0116718, NAR 12(22) 8711 - 87215 1984), particle or microprojectile bombardment (US 5100792,  
20 EP-A-444882, EP-A-434616). microinjection (WO 92/09696, WO 94/00583, EP 331083, EP 175966), electroporation (EP 290395, WO 8706614) or other forms of direct DNA uptake (DE 4005152, WO 9012096, US 4684611).

Agrobacterium transformation is widely used by those  
25 skilled in the art to transform dicotyledonous species. Although Agrobacterium has been reported to be able to transform foreign DNA into some monocotyledonous species (WO 92/14828),

microprojectile bombardment, electroporation and direct DNA uptake are preferred where *Agrobacterium* is inefficient or ineffective. Alternatively, a combination of different techniques may be employed to enhance the efficiency of the transformation process, eg. bombardment with *Agrobacterium* coated microparticles (EP-A-486234) or microprojectile bombardment to induce wounding followed by co-cultivation with *Agrobacterium* (EP-A-486233).

The particular choice of a transformation technology will be determined by its efficiency to transform certain plant species as well as the experience and preference of the person practising the invention with a particular methodology of choice. It will be apparent to the skilled person that the particular choice of a transformation system to introduce nucleic acid into plant cells is not essential to or a limitation of the invention.

The *RPP5* gene, modified versions thereof and related genes encoding a protein showing a significant degree of homology to the protein product of the *RPP5* gene, alleles, mutants and derivatives thereof, may be used to confer pathogen resistance, e.g. to downy mildews, in plants. For this purpose nucleic acid as described above may be used for the production of a transgenic plant. Such a plant may possess pathogen resistance conferred by the *RPP5* gene.

The invention thus further encompasses a host

cell transformed with a vector as disclosed,  
especially a plant or a microbial cell. Thus, a host  
cell, such as a plant cell, comprising nucleic acid  
according to the present invention is provided. Within  
5 the cell, the nucleic acid may be incorporated within  
the chromosome.

A vector comprising nucleic acid according to the  
present invention need not include a promoter, ..  
particularly if the vector is to be used to introduce  
10 the nucleic acid into cells for recombination into the  
genome.

Also according to the invention there is provided  
a plant cell having incorporated into its genome a  
sequence of nucleotides as provided by the present  
15 invention, under operative control of a promoter for  
control of expression of the encoded polypeptide. A  
further aspect of the present invention provides a  
method of making such a plant cell involving  
introduction of a vector comprising the sequence of  
20 nucleotides into a plant cell. Such introduction may  
be followed by recombination between the vector and  
the plant cell genome to introduce the sequence of  
nucleotides into the genome. The polypeptide encoded  
by the introduced nucleic acid may then be expressed.

25 A plant which comprises a plant cell according to  
the invention is also provided, along with any clone  
of such a plant, seed, selfed or hybrid progeny and  
descendants, and any part of any of these, such as

cuttings, seed. The invention provides any plant propagule, that is any part which may be used in reproduction or propagation, sexual or asexual, including cuttings, seed and so on.

5           The invention further provides a method comprising expression from nucleic acid encoding the amino acid sequence SEQ ID NO 2, or a mutant, allele or derivative thereof, or a significantly homologous amino acid sequence, within cells of a plant (thereby  
10           producing the encoded polypeptide), following an earlier step of introduction of the nucleic acid into a cell of the plant or an ancestor thereof. Such a method may confer pathogen resistance on the plant.

          A gene stably incorporated into the genome of a  
15           plant is passed from generation to generation to descendants of the plant, cells of which descendants may express the encoded polypeptide and so may have enhanced pathogen resistance. Pathogen resistance may be determined by assessing compatibility of a pathogen  
20           such as *Peronospora parasitica* or *Bremia lactucae*.

          Sequencing of the *RPP5* gene has shown that like the *Cf-9* gene and the *Cf-2* gene it includes DNA sequence encoding leucine-rich repeat (LRR) regions and homology searching has revealed strong homologies  
25           to other genes containing LRRs. As discussed in WO95/18230, and further validated in this discovery, the presence of LRRs may be characteristic of many pathogen resistance genes and the presence of LRRs can

thus be used in a method of identifying further pathogen resistance genes.

Furthermore, there are some striking homologies between *RPP5* and the tobacco mosaic virus resistance gene *N* and the flax rust resistance gene *L6*. (Figure 3). (As can be derived from Figure 3, the overall homology between *RPP5* and *N* is 33% amino acid identity, while the figure for *RPP5* and *L6* is 27%.) These homologies may also be used to identify further resistance genes, for example using oligonucleotides (e.g. a degenerate pool) designed on the basis of sequence conservation, preferably conservation of amino acid sequence. In particular, primers may be designed that amplify DNA between the regions of the gene that encode the amino acid sequence F Y D V D P (SEQ ID NO 6) of *RPP5* and *N* and where in *L6* it encodes F Y M V D P (SEQ ID NO 7), and the region I A C F F (SEQ ID NO 8) of *RPP5*, where the sequence is identical in *L6* and in *N* is I A C F L (SEQ ID NO 9).

According to a further aspect, the present invention provides a method of identifying a plant pathogen resistance gene comprising use of an oligonucleotide(s) which comprise(s) a sequence or sequences that are conserved between pathogen resistance genes such as *RPP5*, *N* and *L6* to search for new resistance genes. Thus, a method of obtaining nucleic acid comprising a pathogen resistance gene (encoding a polypeptide able to confer pathogen



resistance) is provided, comprising hybridisation of an oligonucleotide (details of which are discussed herein) or a nucleic acid molecule comprising such an oligonucleotide to target/candidate nucleic acid.

- 5 Target or candidate nucleic acid may, for example, comprise a genomic or cDNA library obtainable from an organism known to encode a pathogen resistance gene. Successful hybridisation may be identified and target/candidate nucleic acid isolated for further  
10 investigation and/or use.

Hybridisation may involve probing nucleic acid and identifying positive hybridisation under suitably stringent conditions (in accordance with known techniques) and/or use of oligonucleotides as primers  
15 in a method of nucleic acid amplification, such as PCR. For probing, preferred conditions are those which are stringent enough for there to be a simple pattern with a small number of hybridisations identified as positive which can be investigated  
20 further. It is well known in the art to increase stringency of hybridisation gradually until only a few positive clones remain.

As an alternative to probing, though still employing nucleic acid hybridisation, oligonucleotides  
25 designed to amplify DNA sequences may be used in PCR reactions or other methods involving amplification of nucleic acid, using routine procedures. See for instance "PCR protocols; A Guide to Methods and

Applications", Eds. Innis et al, 1990, Academic Press, New York.

Preferred amino acid sequences suitable for use in the design of probes or PCR primers are sequences  
5 conserved (completely, substantially or partly) between at least two polypeptides able to confer pathogen resistance such as those encoded by *RPP5* and *N* and/or *L6*.

On the basis of amino acid sequence information  
10 oligonucleotide probes or primers may be designed, taking into account the degeneracy of the genetic code, and, where appropriate, codon usage of the organism from the candidate nucleic acid is derived. Preferred nucleotide sequences may include those  
15 comprising or having a sequence encoding amino acids (i) F Y D V D P (SEQ ID NO 6); (ii) I A C F F (SEQ ID NO 8) or a sequence complementary to these encoding sequences. Suitable fragments of these may be employed. For example, the oligonucleotide TTC/T  
20 TAC/T GAC/T GTX GAT/C CC (SEQ ID NO 10) can be derived from the amino acid sequence F Y D V D P. Such an oligonucleotide primer could be used in PCR in combination with the primer A A G/A AA G/A CA XGC T/G/A AT (SEQ ID NO 11), derived from the bottom  
25 strand of the sequence that encodes I A C F F. (All sequences given 5' to 3'; see Figure 3). X indicates A, G, C or T.

Preferably an oligonucleotide in accordance with

the invention, e.g. for use in nucleic acid amplification, has about 10 or fewer codons (e.g. 6, 7 or 8), i.e. is about 30 or fewer nucleotides in length (e.g. 18, 21 or 24).

5           Assessment of whether or not such a PCR product corresponds to resistance genes may be conducted in various ways. A PCR band from such a reaction might contain a complex mix of products. Individual products may be cloned and each one individually screened for  
10 linkage to known disease resistance genes that are segregating in progeny that showed a polymorphism for this probe. Alternatively, the PCR product may be treated in a way that enables one to display the polymorphism on a denaturing polyacrylamide gel and  
15 specific bands that are linked to the resistance gene may be preselected prior to cloning. Once a candidate PCR band has been cloned and shown to be linked to a known resistance gene, it may then be used to isolate cDNA clones which may be inspected for other features  
20 and homologies to either *RPP5*, *N* or *L6*. It may subsequently be analysed by transformation to assess its function on introduction into a disease sensitive variety of the plant of interest. Alternatively, the PCR band or sequences derived by analysing it may be  
25 used to assist plant breeders in monitoring the segregation of a useful resistance gene.

A further method of using the *RPP5* sequence to identify other resistance genes is to use computer

searches of expressed sequence tag (EST) and other DNA sequence databases to identify genes in other species that encode proteins with significant RPP5 homology. For example, a homology score of at least 60 using one  
5 of the BLAST algorithms (Altschul et al, 1990) would indicate a candidate resistance gene.

Having obtained nucleic acid using any of these approaches, a nucleic acid molecule comprising all or part of the sequence of the obtained nucleic acid may  
10 be used in the production of a transgenic plant, for example in order to confer pathogen resistance on the plant.

Modifications to the above aspects and  
15 embodiments and further aspects and embodiments of the present invention will be apparent to those skilled in the art. All documents cited are incorporated herein by reference.

20 Figure 1 shows the genomic DNA sequence of the RPP5 gene (SEQ ID NO. 1). Introns are shown in this Figure in non-capitalised letters. Features: Nucleic acid sequence - Translation start at nucleotide 1966; translation stop at nucleotide 6512.

25 Figure 2 shows predicted RPP5 protein amino acid sequence (SEQ ID NO 2).

Figure 3 shows a comparison of the predicted amino acid sequence of the RPP5 (SEQ ID NO 2), N (SEQ

ID NO 3) and L6 (SEQ ID NO 4) genes. The protein sequences are aligned according to predicted protein domains. Figure 3 was produced using the PRETTYBOX and PileUp programs of the University of Wisconsin Genetics Computer Group Sequence Analysis Software Package Version 7.2.

Figure 4 shows a contiguous nucleotide sequence (SEQ ID NO 5) encoding the amino acid sequence shown in Figure 2 (SEQ ID NO 2), and made by joining together the sequences of the exons of the sequence of Figure 1 (SEQ ID NO 1).

#### Cloning of the *Arabidopsis RPP5* gene

The *RPP5* gene was cloned using a map-based cloning strategy similar in principle to that used for the isolation of the tomato *Pto* gene, described briefly earlier.

##### (i) Assignment of *RPP5* gene map locations

The map location of *RPP5* on the *Arabidopsis* RFLP map has been reported earlier (Parker et al, 1993). This paper describes in detail how two landraces of *Arabidopsis*, designated *Columbia* and *Landsberg erecta*, showed a differential response to a race of *Peronospora parasitica* designated NoCo-2; *Landsberg erecta* is resistant, and *Columbia* is sensitive. Recombinant inbred lines (Lister and Dean, 1993) had been constructed, derived from carrying out single

seed descent on *F2* seed derived from an *F1* between *Landsberg* and *Columbia*, and these recombinant inbred lines were tested for resistance or sensitivity to *NoCo-2*. This analysis showed that the *RPP5* gene lay  
5 on Chromosome 4 between the RFLP markers *m226* and *g4539*. The DNA of *Landsberg* and *Columbia* was analysed using the RAPD (randomly amplified polymorphic DNA) technique (Williams et al, 1990) and polymorphisms between *Landsberg* and *Columbia* were analysed for  
10 linkage to *RPP5*. One polymorphism derived using the operon primer OPC18, which amplified a band in *Columbia* but not in *Landsberg* was absolutely linked to *RPP5*. This DNA band, of 540 bp (referred to as OPC18<sub>540</sub> in Parker et al., 1993) was cloned and the resulting  
15 probe was designated the C18 probe.

(ii) Establishment of a physical map between marker *m226* and marker *g4539*

The *Arabidopsis* genome project has as an  
20 objective the establishment of a physical map of Chromosome 4, and ultimately of the entire *Arabidopsis* genome. The C18 probe was used to identify hybridising yeast artificial chromosome (YAC) clones. This facilitated the establishment of a physical contig  
25 between 4539 and 226 incorporating other linked markers, such as *g13683*. The C18 RAPD band was cloned and used as a probe on *Columbia* and *Landsberg* genomic DNA. Hybridisation of this probe revealed a very

polymorphic small multi-gene family in these two genotypes. Hybridisation to recombinant inbred lines (Lister and Dean, 1993) showed that all members of this multi-gene family were absolutely linked to the resistance gene locus. Using the CAPS procedure (Konieczny and Ausubel, 1993) the individuals in an F2 population derived from selfing an F1 of a *Columbia* and *Landsberg* cross were screened for recombinants between the linked markers Ara-1 and 4539. The primers used for the Ara-1 locus were

Ara-1 5' TCG ACG ACT CTC AAG AAC CC 3'

Ara-2 5' CAC AAG CTA TAC GAT GCT CAC C 3'

This gave a 700 bp band in *Columbia* and *Landsberg* which, after digestion with Acc-1, cut *Landsberg* DNA giving a 360 bp and a 340 bp band. The primers used for the 4539 locus were

4539 F 5' GGT CAT CCG TTC CCA GGT AAA G 3'

4539 R 5' GGA CGT AGA ATC TGA GAG CTC 3'

After Hind III digestion, the *Columbia* 600 bp band remained uncut, whilst the *Landsberg* band was cut to give 480 bp and 120 bp fragments. In this way twenty-

four additional recombinants were derived in this interval. Analysis of these recombinants showed that again all members of the C18 multi-gene family co-segregated exactly with RPP5. Since linked multi-gene families are a characteristic of disease resistance genes (Martin et al, 1993; Jones et al, 1994; Whitham

et al, 1994) we tested the hypothesis that the C18 band might hybridise to the *RPP5* gene. Cosmids were identified from a *Landsberg* binary vector cosmid library in the vector pCLD04541 (C. Dean, pers. com.;  
5 Bent et al, 1994) and cosmid clones that hybridised to the C18 probe were identified. Table 1 lists the hybridising clones. Each of these were used in transformation experiments with the readily transformable *Arabidopsis* landrace, No-O, which is  
10 sensitive to NoC0-2. A transformant was identified derived from transformation with cosmid 29L17, and self-progeny of this transformant segregated for resistance to *P. parasitica* NoC0-2. This demonstrated that the clone 29L17, which carries a band that  
15 hybridises to the C18 RAPD probe, carries a functional *Peronospora parasitica* resistance gene.

(iii) *DNA sequence analysis of the 29L17 plant DNA insert*

20 Cosmid DNA was prepared from 29L17, sonicated and cloned into pUC18 vector and randomly sequenced. Two hundred and forty (240) DNA sequencing reactions were performed on random clones that were identified as clones that hybridised to 29L17 insert DNA, i.e.  
25 clones that carried inserts of plant DNA. From a computer analysis of this DNA sequence data, a DNA sequence contig could be established comprising 14.3 kb of DNA. This DNA sequence was inspected for the



presence of sequences that encoded leucine-rich repeats. One such region, nucleotide 3000 to nucleotide 6138 in SEQ ID NO. 1, was found.

5    (iv) *Analysis of a DNA rearrangement associated with an RPP5 mutation*

One criterion for establishing whether or not a characterised region of plant DNA corresponds to the gene of interest is to inspect whether mutations in  
10    the corresponding gene, caused by ionizing radiation, are associated with DNA rearrangements in the region of interest. Fast neutron mutagenised *Landsberg* seed were screened with *Peronospora parasitica* for mutants to disease sensitivity. Three mutations were found and  
15    analysed by Southern blots for perturbations or rearrangements in DNA corresponding to the gene, carrying leucine rich repeats. One mutant line, FNB387, showed an altered pattern of Southern blot hybridisation. More detailed analysis showed that the  
20    perturbation consists of an insertion of 270 bp of DNA in the C-terminus of the reading frame that carries leucine-rich repeats. Sequence analysis of this region showed that an insertion of 270 bp had arisen from the duplication of several LRRs within the gene carried on  
25    cosmid 29L17. This provides very strong evidence that the *RPP5* gene corresponds to the reading frame that carries leucine-rich repeats.

(v) *Demonstration that a subclone of 29L17 contains RPP5*

To confirm that the gene identified by mutagenesis is not only necessary but also sufficient to confer disease resistance, a subclone of 29L17 was constructed in binary vector SLJ7292. The subclone, designated pRPP5-1, contained a 6304 bp DNA fragment defined by a *Bgl*II restriction enzyme site 5' to the gene (nucleotide 668 in SEQ ID NO. 1) and a *Pst*I restriction enzyme site 3' to the gene (nucleotide 6971 in SEQ ID NO. 1). pRPP5-1 was used to transform *Arabidopsis* ecotype Columbia and shown to confer disease resistance.

(vi) *RT-PCR analysis of the RPP5 transcript*

First strand cDNA was prepared from seedling leaf messenger RNA and PCR amplification from this cDNA was performed using intron flanking primers. The primers were: for intron 1, 5'-GAGTTCGCTCTATCATCTCC and 5'-TTATTGCATTGCGAAACATCATTG; for introns 2 and 3, 5'-AAATTGATCGTGCAAAGTCC and 5'-AAGATTCGCATTCTTCAAGATT; for intron 4, 5'-GAAGATGGATTTGTATAATTCC and 5'-TCAAATTCGGGCATCCAGTG. For intron 5 a nested PCR strategy was employed, an aliquot of the products of the first amplification being used as the template for the second. The primers used were: for the first amplification, 5'-TGGTGACACTTCCTTCCTCG and 5'-CCAAACTTTTGCAAGTTGTTG; for the second amplification

5'-TCTCAATGTGAGCGGCTGCAAGC and  
5'-AACTTGAGCAACCACTGAGATCG. Cloned PCR products were  
sequenced using a combination of vector-specific and  
insert-specific primers. Intron sequences are shown in  
5 lower case in SEQ ID NO. 1 (Figure 1) between the exon  
sequences shown in upper case.

*(vii) Comparison of the RPP5 gene sequence with the  
sequences of other resistance genes*

10        Comparison of the RPP5 sequence to the genes *N*  
and *L6* reveals very strong homologies throughout the  
*N*-  
terminal region. These regions are highlighted in  
Figure 3. They include regions involved in nucleotide  
15 binding, designated Kinase-1a, Kinase-2, Kinase-3a.  
Kinase-1a is often referred to as the P-loop. Also,  
regions N-terminal to the nucleotide binding domain  
show conspicuous homologies. Primers were designed  
particularly to the conserved regions carrying the  
20 amino acid sequence F Y D V D P (amino acids 104 to  
109 of RPP5) and to amino acids I A C F F (437-441 of  
RPP5). When degenerate oligonucleotide primers based  
on amino acid sequence were used in PCR reactions,  
both on *Arabidopsis* genomic DNA and on cDNA made from  
25 RNA of other species, products were observed of the  
size consistent with the potential to encode  
resistance genes.

These primers could alone, or in combination with

other primers encoding conserved and non-conserved regions of the identified resistance genes, be used to isolate other homologous gene sequences which could include previously uncharacterized resistance genes.

## Table 1:

Binary vector cosmid clones hybridising to C18

Binary vector: 04541

5 Transformed into No-o

3D23

27E2

29L17

38G10

10 42P15

Subsequently identified:

45F8

18A10

56G2

36

Table 2:

% amino acid identity between RPP5 and N; RPP5 and L6

	N exon1	N exon 2	N exon 3	N exon 4+5
5				
RPP5				
exon 1	55			
exon 2		36		
exon 3			26	
10 exon 4,5+6				26

	L6 exon 1	L6 exon 2	L6 exon 3	L6 exon 4
15				
RPP5				
exon 1	37			
exon 2		30		
exon 3			17	
exon 4,5+6				26

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CLAIMS

1. A nucleic acid isolate encoding a pathogen resistance gene whose expression in a plant can cause activation of a defence response in the plant,  
5 comprising a sequence of nucleotides encoding a polypeptide comprising the sequence of amino acids shown in Figure 2
2. Nucleic acid according to claim 1 wherein said  
10 activation is upon contact of the plant with a pathogen or corresponding elicitor molecule.
3. Nucleic acid according to claim 1 wherein the sequence of nucleotides comprises an encoding sequence  
15 shown in Figure 1.
4. Nucleic acid according to claim 1 wherein the sequence of nucleotides comprises an allele, derivative or mutant, by way of addition, insertion,  
20 deletion or substitution of one or more nucleotides, of an encoding sequence shown in Figure 1.
5. Nucleic acid encoding a pathogen resistance gene whose expression in a plant can cause activation of a  
25 defence response in the plant, comprising a sequence of nucleotides encoding a polypeptide, the polypeptide comprising an amino acid sequence which comprises an allele, derivative or mutant, by way of addition,

insertion, deletion or substitution of one or more amino acids, of the amino acid sequence shown in Figure 2;

with the proviso that the encoded polypeptide has  
5 at least about 60% homology with the amino acid sequence shown in Figure 2.

6. Nucleic acid encoding a pathogen resistance gene whose expression in a plant can cause activation of a  
10 defence response in the plant, comprising a sequence of nucleotides encoding a polypeptide, the polypeptide comprising an amino acid sequence which comprises an allele, derivative or mutant, by way of addition, insertion, deletion or substitution of one or more  
15 amino acids, of the amino acid sequence shown in Figure 2;

with the proviso that expression of the nucleic acid can cause said activation of a defence response upon contact of the plant with an Oomycete fungus,  
20 such as *Peronospora parasitica*, or an extract thereof.

7. Nucleic acid according to claim 5 or claim 6 wherein said activation is upon contact of the plant with a pathogen or corresponding elicitor molecule.  
25

8. Nucleic acid which is a vector comprising nucleic acid according to any one of claims 1 to 7.

9. Nucleic acid according to claim 8 further comprising regulatory sequences for expression of said polypeptide.

5 10. Use of nucleic acid according to any one of the precedings claims in production of a transgenic plant.

11. A host cell comprising nucleic acid according to any one of claims 1 to 9.

10

12. A host cell according to claim 11 which is microbial.

13. A host cell according to claim 11 which is a  
15 plant cell.

14. A plant or any part thereof comprising a cell according to claim 13.

20 15. Seed, selfed or hybrid progeny or a descendant or derivative or extract of a plant according to claim 14, or any part thereof.

16. A method which comprises introduction of nucleic  
25 acid according to any one of claims 1 to 9 into a host cell.

17. A method according to claim 16 wherein the host

cell is a plant or microbial cell.

18. A method of conferring pathogen resistance on a plant, comprising expression from nucleic acid  
5 according to any one of claims 1 to 9, within cells of the plant, following an earlier step of introduction of the nucleic acid into a cell of the plant or an ancestor thereof.
- 10 19. A method according to claim 18 wherein the nucleic acid encodes an amino acid sequence shown in Figure 2.
- 15 20. An oligonucleotide comprising a sequence encoding an amino acid sequence conserved between RPP5 of *Arabidopsis* and another pathogen resistance genes or comprising a sequence complementary to a nucleotide sequence encoding a said amino acid sequence.
- 20 21. An oligonucleotide according to claim 20 wherein the pathogen resistance gene is *N* of tobacco or *L6* of flax.
- 25 22. An oligonucleotide according to claim 21 comprising a nucleotide sequence encoding one of the amino acid sequences:

(i)

F Y D/M V D P; and

(ii)

I A C F F/L

or comprising a nucleotide sequence complementary to a said encoding sequence.

5

23. An oligonucleotide according to claim 22 comprising a sequence selected from:

(i)

T T C/T T A C/T G A C/T G T X G A T/C C C;

10 (ii)

A A G/A A A G/A C A X G C T/G/A A T; and

(iii)

a sequence complementary to (i) or (ii).

15 24. An oligonucleotide which comprises a sequence which is a variant or derivative, by way of addition, insertion, deletion or substitution of one or more nucleotides, of the sequence of an oligonucleotide according to any one of claims 20 to 23.

20

25 25. A method of obtaining nucleic acid comprising a pathogen resistance gene comprising hybridisation of an oligonucleotide according to any one of claims 20 to 24, or a nucleic acid molecule comprising a said oligonucleotide, to target nucleic acid.

26. A method according to claim 25 involving use of nucleic acid amplification.

27. A method according to claim 25 or claim 26 wherein the hybridisation is followed by identification of successful hybridisation and isolation of target nucleic acid.

5

28. A method wherein following the obtaining of nucleic acid using the method of any of claims 25 to 27 a nucleic acid molecule comprising all or part of the sequence of the obtained nucleic acid is used in  
10 the production of a transgenic plant.

29. A method according to claim 28 wherein said nucleic acid molecule is used to confer pathogen resistance on said plant.

Figure 1

```

1   atgtgaccag cacaaggaac aacccttcag gtgaaaagag aagagagcct
51  ttgccatgtc atggaagaaa agcgctttta gattttctgc aagagacaat
101 gtctgttcca attccaaacc atgaagtatc atccaaagct cctacgtcta
151 tgagaaaacg ggtagctgct cttccagggg aagctgagaa ggaacttctt
201 tatctgacct caatgccact gtgctctaac ggtcggcctg aagcaggtgc
251 agttctgggtg taatcattga cttttgtttt tgtttcagag ttttaatttaa
301 accttaagaa gagtttttct gactttacta ggggacgtaa ttgagaagaa
351 agacggtcct tgctcaggaa ccaaaggctt ccgagctcca gaggtacatc
401 aactgtaact atagctcact tctcttaata cgagttaaat atgaaaatct
451 tgtgccttca gactaaagtt gtaggcctga gctactcatt agcacatctc
501 tgatgggaaa gctaattctc tggggctggt cttggctgaa ttggtttgtg
551 caggtttgct tcagatcttt gcaccaagga cctaagatag acgtgtggtc
601 tgcgggagtt actttgttat acctcataat gggaaggaca cctttcactg
651 gtgaccctga acagttaaga tctgacttga atgagtacac catcactcgg
701 ttccactcgt ttcttctttt ttgccttctt acaacgacct tgtatttccc
751 aggaacataa aggacattgc acaactacga ggcagtgaag aattatggga
801 agtagccaag ctgcacaacc gtgaatcctc tttccctaag gtaacatctc
851 atcttaccac tcttctgtac tctccattca tttatgagac acatatactt
901 gctctgttct ttttaccgcc agcttaaata tcacataaaa gggttttcat
951 tgttagcggt ttgtgttcta ttgcttctac tgcaactcca ttacagaaca
1001 tatatatctt aataatgtaa attgatgaca aagtgattaa atagatgatc
1051 gtgagagatg aaatcaggta gagttttgtg ttgttgtttc aggaattata
1101 cgagtcaagg tacttgaagg ggatggagtt gagaaaatgg ggcgaacgca
1151 acacaaaaag cagagagttt ctagacgcaa ttccacggcc gcttcttgaa
1201 ctcgttgata gatgtttgat agttaaccgg aggcgacgaa tcagcgcaga
1251 ggatgctctc aagcacgagt tcttctatcc agtacatgaa acccttagaa
1301 accaaatgct ccttaaacag cagcaaatgc aatcgcagcc tacagttggt
1351 gctgacgcac taagcgaaac tttaaactaa ttatacaatt cttaaaaact
1401 aaaagagtaa tttagcaaac tagagagtta attttcactt tagcaaaacta
1451 gagagttaat ttaatttagc gaactaatta tattttcact ttagtataca
1501 attcttagtg ttaatttagt attttcactt atattatttg aattaaaatc
1551 ctcataatcg atatacttat tctcctaata catgtgcatg tatgtattgg
1601 gaaacaagac tttgatatta aacaatcata agtacattct tacgataaaa
1651 tgtcttgtag aaggacaact gacaccacaa aaatatgtgt gtttcaaaat
1701 atctgtgtag aggaaacgaa tgtaagtttc tgtctaattg cctagaactt
1751 gaaatattat ttctgtcttg taaaaagact aagacttatc ataattaagt
1801 gacaaccaca aaaattcaat ctctaaaaat atctttgtat gtagtgtaaa
1851 aaagctttcg aggaaagtaa gacgaagttt ctctctctct tctcacacta
1901 tgtcttgctg atttacttct cttaaaaatc ttcgtctctt ctctgagttc
1951 gctctatcat ctcccATGGC GGCTTCTTCT TCTTCTGGCA GACGGAGATA
2001 CGACGTTTTT CCAAGCTTCA GTGGGGTTGA TGTTGCAAG ACGTTCCTCA
2051 GCCATCTTCT CAAGGCTCTC GACGGCAAAT CAATCAATAC ATTCATCGAT
2101 CATGGAATCG AGAGAAGCCG CACAATCGCC CCTGAGCTTA TATCGGCGAT
2151 TAGAGAAGCT AGGATCTCAA TCGTCATCTT CTCTAAGAAC TATGCTTCTT
2201 CAACGTGGTG CTTAAATGAA TTGGTTGAGA TCCACAAGTG CTTTAATGAT
2251 TTAGGTCAAA TGGTGATTCC AGTTTTCTAC GACGTTGATC CTTCGGAAGT
2301 TAGAAAACAG ACCGGCGAAT TTGGAAAGGT CTTTGAAAAG ACATGCGAGG
2351 TCAGCAAGGA CAAACAACCA GGGGATCAGA AACAAAGATG GGTGCAAGCT
2401 CTCACAGATA TAGCAAATAT AGCCGGAGAG GATCTTCTGA ACGGgtacgt
2451 tgttatgatt ccaatatatc tgcttgcggt ttcaattgtc tcagaactat
2501 atttttgcat agacttcggt tcttctttta ggggtgcttc ttaattgaca
2551 aaattgactt ttgttattag GCCTAATGAA GCGCATATGG TTGAAAAGAT

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Figure 1 (continued)

2601	ATCCAATGAT	GTTTCGAATA	AACTTATCAC	TCGGTCAAAG	TGTTTTGATG
2651	ACTTCGTCGG	AATTGAAGCT	CATATTGAGG	CAATAAAATC	AGTATTGTGC
2701	TTGGAATCCA	AGGAAGCTAG	AATGGTCGGG	ATTTGGGGAC	AGTCAGGGAT
2751	TGGTAAGAGT	ACCATCGGAA	GAGCTCTTTT	CAGTCAACTC	TCTAGCCAGT
2801	TCCACCATCG	CGCTTTCCTA	ACTTATAAAA	GCACCAGTGG	TAGTGACGTC
2851	TCTGGCATGA	AGTTGAGTTG	GCAAAAAGAG	CTTCTCTCGG	AAATCTTAGG
2901	TCAAAAGGAC	ATAAAGATAG	AGCATTTTGG	TGTGGTGGAG	CAAAGGTTAA
2951	ATCACAAGAA	AGTTCTTATC	CTTCTTGATG	ATGTGGATAA	TCTAGAGTTT
3001	CTTAAGACCT	TGGTGGGAAA	AGCTGAATGG	TTTGGATCTG	GAAGCAGAAT
3051	AATTGTGATC	ACTCAAGATA	GGCAACTTCT	CAAGGCTCAT	GAGATTGACC
3101	TTGTATATGA	GGTGAAGCTG	CCATCTCAAG	GTCTTGCTCT	TAAGATGATA
3151	TCCAATATG	CTTTTGGGAA	AGACTCTCCA	CCTGATGATT	TTAAGGAACT
3201	AGCATTTGAA	GTTGCCGAGC	TTGTCGGTAG	TCTTCCTTTG	GGTCTCAGTG
3251	TCTTGGGTTC	ATCTTTAAAA	GGAAGGGACA	AAGATGAGTG	GGTGAAGATG
3301	ATGCCTAGGC	TTCGAAATGA	TTCAGATGAT	AAAATTGAGG	AAACACTAAG
3351	AGTCGGCTAC	GATAGGTTAA	ATAAAAAAAA	TAGAGAGTTA	TTTAAGTGCA
3401	TTGCATGTTT	TTTCAATGGT	TTTAAAGTCA	GTAACGTCAA	AGAATTACTT
3451	GAAGATGATG	TTGGGCTTAC	AATGTTGGCT	GAGAAGTCCC	TCATACGTAT
3501	TACACCGGGT	GGATATATAG	AGATGCACAA	TTTGCTAGAG	AAATTGGGTA
3551	GAGAAATTGA	TCGTGCAAAG	TCCAAGGGTA	ATCCTGGAAA	ACGTCAATTT
3601	CTGACGAATT	TTGAGGATAT	TCGAGAAGTA	TTGACCGAGA	AAACTgtaag
3651	tttttcgcat	ctccttaaac	gttgtaatgc	atgactttat	atcaatataa
3701	tcgtaatttg	gggattgata	aacttaagca	attggtgccc	catgcgtaat
3751	taaaacgtag	ctttgatgtg	tcagaaaaat	aaaaagggtt	gcgattgtta
3801	agattatatt	agttttcttc	ggattttttt	tcagGGGACC	GAAACTCTTC
3851	TTGGAATACG	TTTGCCACAC	CCGGGATATC	TTACGACAAG	GTCGTTCTTA
3901	ATAGATGAAA	AATCATTCAA	AGGCATCGGT	AATCTCCAAT	ATCTAGAAAT
3951	TGGTTATTGG	TCAGATGGGG	TTCTACCTCA	GAGCCTCGTT	TATTTCCCTC
4001	GTAAACTCAA	AAGGCTATGG	TGGGATAATT	GTCCATTGAA	GCGTTTGCCT
4051	TCTAATTTTA	AGGCTGAGTA	TCTGGTTGAA	CTCAGAATGG	TGAATAGTAA
4101	GCTTGAGAAG	CTGTGGGATG	GAACTCAGgt	actaattttt	ttagtgatca
4151	atttctaaac	ataaaaaacta	aaaataaaaa	tgtttaaaat	gttcattaac
4201	gtgtgtgctc	tcttttcccc	tattttgttt	tcagCCCCTT	GGAAGTCTCA
4251	AGAAGATGGA	TTTGTATAAT	TCCTACAAAT	TGAAAGAAAT	TCCAGATCTT
4301	TCTTTAGCCA	TAAACCTCGA	GGAATTAAAT	CTTGAAGAAT	GCGAATCTTT
4351	GGAGACACTT	CCTTCCTCGA	TTCAGAATGC	CATTAAACTG	AGGGAGTTAA
4401	ATTGTTGGGG	GGGGCTATTA	ATAGATTTAA	AATCATTAGA	AGGCATGTGT
4451	AATCTCGAAT	ATCTATCAGT	TCCTAGTTGG	TCAAGTAGGG	AATGCACTCA
4501	GGGCATCGTT	TATTTCCCTC	GTAAACTCAA	AAGTGTATTG	TGGACTAATT
4551	GTCCATTGAA	GCGTTTGCCT	TCTAATTTTA	AGGCTGAGTA	TCTGGTTGAA
4601	CTCATAATGG	AGTACAGTGA	GCTTGAGAAG	CTGTGGGATG	GTA CT CAGgt
4651	actaattcta	ttagtataa	taaatatgtt	agaaaaacta	aaaataaaaa
4701	tgtttaaaat	gttcattaac	gtgtgtgctc	tcttttcccc	tattttgtta
4751	tcagTCACTT	GGAAGTCTCA	AGGAGATGAA	TTTGAGGTAT	TCCAACAATT
4801	TAAAAGAAAT	TCCAGATCTT	TCTTTAGCCA	TAAACCTCGA	GGAATTAGAT
4851	CTTTTTGGAT	GCGTATCTTT	GGTGACACTT	CCTTCCTCGA	TTCAGAATGC
4901	CACTAAACTG	ATCTATTTAG	ATATGAGTGA	ATGCGAAAAT	CTAGAGAGTT
4951	TTCCAACCGT	TTTCAACTTG	AAATCTCTCG	AGTACCTCGA	TCTCACTGGA
5001	TGCCCCGAAT	TGAGAAATTT	CCCAGCAATC	AAAATGGGAT	GTGCCTGGAC
5051	TAGATTATCT	CGAACAAGAT	TGTTTCCGGA	AGGGAGAAAT	GAGATCGTGG
5101	TAGAAGATTG	TTTCTGGAAC	AAGAATCTCC	CTGCTGGACT	AGATTATCTC
5151	GACTGCCTTA	TGAGATGTAT	GCCTTGTGAA	TTTCGCTCAG	AACAATCAC



Figure 1 (continued)

5201	TTTTCTCAAT	GTGAGCGGCT	GCAAGCTTGA	GAAGCTATGG	GAAGGCATCC
5251	AGgtacattg	ttaatgctat	gctgattttt	gtttaccttc	tgttatataa
5301	ctaattaagt	atacccaaat	ttgtttttat	ggcttggtgt	cgatccacgg
5351	ttatgtctta	catacataca	taataatggt	taattataat	tttaaacata
5401	tatagggtata	aaattaaaaat	gattatcatc	gataatgatt	gaagcatacc
5451	aatgtttttt	tcagTCGCTT	GGAAGTCTCG	AAGAGATGGA	TCTGTCAGAA
5501	TCTGAAAACC	TGAAAGAACT	TCCAGATCTT	TCAAAGGCCA	CCAATCTGAA
5551	GCTTTTATGT	CTCAGCGGGT	GCAAAAGTTT	GGTGACACTT	CCTTCTACAA
5601	TTGGGAATCT	TCAAAATTTG	AGACGTTTGT	ACATGAACAG	ATGCACAGGG
5651	CTGGAGGTTT	TTCCGACCGA	TGTCAACTTG	TCATCTCTCG	AAACCCTCGA
5701	TCTCAGTGGT	TGCTCAAGTT	TGAGAACTTT	TCCTCTGATT	TCAACTAATA
5751	TTGTATGTCT	CTATCTGGAA	AACACCGCCA	TTGAAGAAAT	TCCAGATCTT
5801	TCAAAGGCCA	CCAAGCTCGA	GTCTTTGATA	CTCAACAACT	GCAAAAGTTT
5851	GGTGACACTT	CCTTCTACAA	TTGGGAATCT	TCAAAATTTG	AGACGTTTGT
5901	ACATGAACAG	ATGCACAGGG	CTGGAGCTTC	TTCCGACCGA	TGTCAACTTG
5951	TCATCTCTCG	AAACCCTCGA	TCTCAGTGGT	TGCTCAAGTT	TGAGAACTTT
6001	TCCTCTGATT	TCAACTAGAA	TCGAATGTCT	CTATCTAGAA	AACACCGCCA
6051	TTGAAGAAGT	TCCCTGCTGC	ATTGAGGATT	TCACGAGGCT	CACTGTACTA
6101	CGGATGTATT	GTTGCCAGAG	GTTGAAAAC	ATCTCCCCAA	ACATTTTCAG
6151	ACTGACTAGT	CTTACGCTCG	CCGACTTTAC	AGACTGTAGA	GGTGTATCA
6201	AGGCGTTGAG	TGATGCAACT	GTGGTAGCGA	CAATGGAAGA	TCACGTTTCT
6251	TGTGTACCAT	TATCTGAAAA	CATTGAATAT	ACATGTGAAC	GTTTCTGGGA
6301	TGCGTGTTCT	GATTATTACT	CTGATGACTT	TGAGGTAAAT	CGGAACCCAA
6351	TTAGATTGTC	AACGATGACT	GTCAACGATG	TGGAGTTTAA	GTTTTGTTGC
6401	TCCATTACGA	TCAAAGAATG	CGGTGTACGA	CTCTTGTATG	TCTATCAAGA
6451	AACAGAGCAC	AACCAACAAA	CTACGAGAAG	CAAGAAGCGG	ATGCGGGTAA
6501	GCCTTTTGCC	Ataattagag	ctgaaacttg	taaagcaatc	ttttgacttg
6551	atttgtttta	taggatcaaa	ataccatagc	gacagactat	ttgatagaat
6601	cgatcgtttg	atatataatg	cagatgacat	cggggacatc	tgaagaagat
6651	atcaacttac	cctatggcca	aattgtagcg	gacacaggat	tggccgctct
6701	aaatacacag	ctttcgttag	ggcagggaga	agcatcatca	tcaacatctc
6751	tagaggggga	agctttgtgt	gttgatgatt	acatgataaa	tgaagaacaa
6801	gatgaacaaa	tacctatctt	gtatcctggt	tatggtaact	gaagcatctt
6851	tatcattctg	ttttgctctt	ttttaggata	acttgggatc	gaccattatt
6901	ataaatttat	aatgataatg	acaaaacgat	ttcataggtt	ttgacttttg
6951	acacaagcca	ttttttctgc	agatatagac	gatgatatgt	ggagatcatt

Figure 2.

1 MAASSSSGRR RYDVFPSPFSG VDVRKTFLSH LLKALDGKSI NTFIDHGIER  
51 SRTIAPELIS AIREARISIV IFSKNYASST WCLNELVEIH KCFNDLGQMV  
101 IPVFYDVPDS EVRKQTGEFG KVFEKTCEVS KDKQPGDQKQ RWVQALTDIA  
151 NIAGEDLLNG PNEAHMVEKI SNDVSNKLIT RSKCFDDFVG IEAHIEAIKS  
201 VLCLESKEAR MVGIWGQSGI GKSTIGRALF SQLSSQFHHR AFLTYKSTSG  
251 SDVSGMKLSW QKELLSEILG QKDIKIEHFG VVEQRLNHKK VLILLDDVDN  
301 LEFLKTLVGK AEWFGSGSRI IVITQDRQLL KAHEIDLVEY VKLPSQGLAL  
351 KMISQYAFGK DSPPDDFKEL AFEVAELVGS LPLGLSVLGS SLKGRDKDEW  
401 VKMMPRLRND SDDKIEETLR VGYDRLNKKK RELFKCIACF FNGFKVSNVK  
451 ELLEDDVGLT MLAEKSLIRI TPGGYIEMHN LLEKLGREID RAKSKGNPGK  
501 RQFLTNFEDI REVLTEKTGT ETLLGIRLPH PGYLTTRSFL IDEKSFKGMR  
551 NLQYLEIGYW SDGVLPQSLV YFPRKLKRLW WDNCPKRLP SNFKAEYLVE  
601 LRMVNSKLEK LWDGTQPLGS LKKMDLYNSY KLKEIPDLISL AINLEELNLE  
651 ECESLETLP SIONAIKLRE LNCWGGLLID LKSLEGMCNL EYLSVPSWSS  
701 RECTQGIVYF PRKLKSVLWT NCPLKRLPSN FKAEYLEVELI MEYSELEKLW  
751 DGTQSLGSLK EMNLRYSNL KEIPDLISLAI NLEELDLFGC VSLVTLPSI  
801 QNATKLIYLD MSECENLESF PTVFNLSLE YDLTGCPNL RNFPKMGK  
851 AWTRLSRTRL FPEGRNEIVV EDCFWNKNLP AGLDYLDCLM RCMPCEFRSE  
901 QLTFLNVSGC KLEKLWEGIQ SLGSLEEMDL SESENKELP DLSKATNLKL  
951 LCLSGCKSLV TLPSTIGNLQ NLRRLYMNRC TGLEVLPTDV NLSSLETLDL  
1001 SGCSSLRFTP LISTNIVCLY LENTAIEEIP DLSKATKLES LILNNCKSLV  
1051 TLPSTIGNLQ NLRRLYMNRC TGLELLPTDV NLSSLETLDL SGCSSLRFTP  
1101 LISTRIECLY LENTAIEEVP CCIEDFTRLT VLRMYCCQRL KNISPNIFRL  
1151 TSLTLADFTD CRGVIKALSD ATVVATMEDH VSCVPLSENI EYTCERFWDA  
1201 CSDYYSDDFE VNRNPIRLST MTVNDVEFKF CCSITIKECG VRLLYVYQET  
1251 EHNQQTTRSK KRMRVSLLP



Figure 3 (continued)

335 R L P H 2 G Y L T T  
 N W V . S S Y S S T  
 L6 S I P W G V . . .  
 R S F L I D E K S E  
 . . L R F S N Q A V  
 . K Y E F K S E C E  
 R G V R N N L Q Q Y L E  
 R N V K R R L R V R N  
 L N B S E F R Y L H  
 I G Y H S D G V L P  
 M G R S S T H Y . .  
 A . . R E A M L T  
 Q S L V Y R R K L V  
 . A I D Y L P N N L  
 G D F E N N L L P N L  
 K R R L . . H W D N  
 K R C E . . V C T N  
 K R H L E L P F Y K H  
 583  
 583  
 638

[illegible][illegible]

305 **Z** **E** **G** **H** **C** **N** **L** **E** **Y** **L** **S** **V** **P** **S** **W** **S** **R** **E** **C** **T** **Q** **G** **I** **V** **Y** **F** **P** **R** **K** **L** **K** **R** **L** **E** **S** **N** **F** **K** **A** **E** **Y** **L** **V** **E** **L** **E** **M** **E** **Y** 743  
 N **L** **K** . . . **R** **E** **P** **C** **V** **N** **V** **E** **S** . . . . . **E** **Y** **L** **G** . . . **A** **R** **S** **C** 698  
 35 **L** **R** **E** **L** **C** . **L** **E** **F** **N** **H** **G** **T** **N** **L** **R** **E** **V** **V** **A** **D** **I** **G** **Q** **L** **S** **S** **L** **K** **V** **L** **K** **T** **T** **G** **A** **K** **E** **V** **E** **I** **N** **E** **F** **L** **G** **L** **K** **E** **L** **S** **T** **S** **S** **R** **I** **P** **N** **L** 812

6/9

798	754	870
GCUSVUTPS	NMKVIAAPS	INTVNUDDIAS
...F	...H	EKTR
EEEDU	VTKLL	EKSKQL
PD..LSLAIN	ESSIFQYKTH	ESSVHWKVSK
IRYSNNLK	WQ.GSGIRCL	WPPASP.SSD
QSLGSLKEMN	GRMKPEIQIH	KVYDCKDGED
GT	IY	EVL
WGT	PEIY	EVL
SEGERU	SEGERU	SQMLDL
NDS	SEGERU	SEGERU
SE	SE	SE

255 **S I Q N A** . . . . . **A K L I Y T D M S** **V F N T K S T E Y T** **Q L** . . . . . **T G C P N T R N E P** 844  
 N **S I C R T** . . . . . **K S L V S L S V S** **G C S K L E S T P E** **E I** . . . . . **G D L D N T R V E D** 790  
 16 **S G G H L P R Y L L** **P T S L T Y L K I Y** **Q C T E P T H L P G** **I E N T E N T S L** **E V N D I F Q T L G** **G D L D G L Q G R** 930

ps5 A I K M G C A N T R L S R T R T F P E G R N E I V V E D C F W N K N H . . . . B A G L D Y D D C L M R C M P C E F R S 899  
N A S D T U I L R P P S S I R T . . . L M F R G F . . . K D G V H F . . . F F P P V A E G L H S 834  
L6 S L E I L R I R K V N G L A R X . . K G L K D M L C S S T C K L R K F Y I T E C P D L I E L T P C E L G V Q T V V V P S 988

[illegible]

35 CLSGCKSLVT  
 N . . . . FBH  
 35 LDDTSSGIER  
 PRITGNLON  
 PRSSIA . . .  
 TVSS . SKLOK  
 LRRLYNNRCT  
 . . . . .  
 LTTVV . KVP  
 GEVLPTDVN  
 . . . . . Q  
 SREIEGLEE  
 LSSGLTLDJS  
 L GALOSLDLK  
 L KSTODLYE  
 GCSTRTBPL 1011  
 CCQRLTQPE 899  
 GCTSLGRTEJ 1104

[illegible]

Figure 3 (continued)

Rpps N L6	<p> <b>T</b>Y<b>M</b>N<b>R</b>C<b>T</b><b>G</b><b>L</b><b>E</b>  <b>L</b>F<b>A</b>...<b>Y</b><b>F</b><b>M</b><b>E</b><b>O</b>  <b>L</b>6...<b>N</b><b>I</b><b>T</b><b>K</b><b>E</b><b>D</b><b>E</b><b>L</b><b>E</b> </p>	<p> <b>L</b>P<b>T</b>D<b>V</b><b>N</b><b>I</b><b>S</b><b>S</b>  ...<b>N</b><b>I</b><b>S</b><b>S</b>  <b>V</b><b>G</b><b>S</b><b>L</b><b>E</b><b>E</b><b>L</b><b>D</b><b>S</b> </p>	<p> <b>L</b>E<b>T</b><b>L</b><b>D</b><b>L</b><b>S</b><b>G</b><b>C</b><b>S</b>  <b>M</b>R<b>H</b>...<b>D</b><b>I</b><b>S</b><b>A</b><b>S</b><b>D</b>  <b>L</b>E<b>L</b><b>T</b><b>L</b><b>D</b><b>D</b><b>T</b><b>C</b><b>S</b> </p>	<p> <b>S</b>L<b>R</b><b>T</b><b>F</b><b>P</b><b>M</b><b>I</b><b>S</b>  <b>S</b>L<b>S</b><b>L</b><b>T</b><b>V</b><b>E</b><b>T</b><b>G</b><b>Q</b>  <b>S</b>H<b>E</b><b>R</b><b>I</b><b>S</b><b>E</b><b>L</b><b>S</b><b>K</b> </p>	<p> ...<b>T</b>R<b>E</b><b>C</b><b>L</b><b>Y</b>  <b>P</b><b>Y</b><b>P</b><b>E</b><b>R</b><b>I</b><b>P</b><b>S</b><b>M</b><b>F</b>  ...<b>L</b><b>O</b><b>R</b><b>D</b><b>T</b><b>T</b><b>L</b><b>I</b> </p>	<p> <b>L</b>E<b>N</b><b>T</b><b>A</b><b>I</b><b>E</b><b>V</b><b>P</b>  <b>H</b><b>H</b><b>Q</b><b>G</b><b>W</b><b>D</b><b>S</b><b>S</b><b>V</b><b>S</b>  <b>V</b><b>E</b><b>V</b><b>P</b><b>S</b><b>L</b><b>R</b><b>E</b><b>R</b> </p>	<p> 1120 994 1221 </p>
Rpps N L6	<p> <b>C</b>C<b>I</b><b>S</b><b>D</b><b>E</b>...  <b>V</b>N<b>L</b>P<b>E</b>N<b>W</b><b>X</b><b>I</b><b>P</b>  <b>E</b>G<b>L</b><b>A</b><b>E</b>L </p>	<p> <b>T</b>R<b>T</b><b>V</b><b>L</b><b>R</b><b>M</b><b>Y</b><b>C</b>  <b>D</b>K<b>K</b><b>L</b><b>G</b><b>E</b><b>A</b><b>V</b><b>C</b><b>Y</b>  <b>K</b>S<b>L</b><b>R</b><b>I</b><b>L</b><b>Y</b><b>L</b><b>E</b><b>G</b> </p>	<p> <b>C</b>O<b>R</b><b>L</b><b>K</b><b>N</b><b>I</b><b>S</b><b>E</b><b>N</b>  <b>C</b>S<b>R</b><b>S</b><b>L</b><b>I</b><b>D</b><b>T</b><b>T</b><b>A</b><b>H</b>  <b>C</b>T<b>S</b><b>L</b><b>E</b><b>R</b><b>L</b><b>W</b><b>E</b><b>D</b> </p>	<p> <b>I</b>F<b>R</b><b>L</b><b>T</b><b>S</b><b>L</b><b>T</b><b>L</b><b>A</b>  <b>L</b>I<b>P</b><b>M</b><b>C</b><b>D</b><b>D</b><b>K</b><b>M</b><b>S</b>  <b>Q</b><b>Q</b><b>Q</b><b>L</b><b>G</b><b>S</b><b>L</b><b>K</b><b>N</b><b>L</b> </p>	<p> <b>D</b>E<b>T</b><b>D</b><b>C</b><b>R</b><b>G</b><b>V</b><b>I</b><b>K</b>  <b>R</b>M<b>T</b><b>G</b>...<b>I</b><b>Q</b>  <b>N</b>V<b>L</b><b>D</b><b>I</b><b>Q</b> </p>	<p> <b>A</b>L<b>S</b><b>D</b><b>A</b><b>T</b><b>V</b><b>A</b><b>T</b>  ...<b>C</b>K<b>S</b><b>L</b><b>S</b><b>V</b><b>D</b><b>H</b> </p>	<p> 1176 1038 1272 </p>
Rpps N L6	<p> <b>M</b>E<b>D</b><b>H</b><b>V</b><b>S</b><b>C</b><b>V</b><b>E</b><b>L</b>  ...<b>K</b><b>L</b><b>A</b><b>L</b>  <b>L</b>S<b>A</b>L<b>K</b><b>T</b><b>T</b><b>L</b><b>P</b> </p>	<p> <b>S</b>E<b>N</b><b>E</b><b>X</b><b>T</b><b>C</b><b>E</b><b>R</b>  <b>S</b>E...<b>C</b>D<b>T</b><b>E</b><b>S</b><b>S</b><b>N</b>  <b>R</b>A<b>R</b>E<b>T</b><b>W</b><b>P</b><b>D</b><b>Q</b><b>P</b> </p>	<p> <b>W</b>D<b>A</b><b>C</b><b>S</b><b>D</b><b>Y</b><b>S</b>  <b>Y</b><b>S</b><b>E</b><b>H</b><b>D</b><b>I</b><b>H</b><b>F</b><b>F</b>  <b>Y</b><b>R</b>... </p>	<p> <b>D</b>D<b>E</b><b>V</b><b>N</b><b>R</b><b>N</b><b>P</b><b>I</b>  <b>V</b><b>P</b><b>E</b><b>A</b><b>G</b><b>L</b><b>W</b><b>D</b><b>T</b><b>S</b>  ... </p>	<p> <b>R</b>L<b>S</b><b>T</b><b>M</b><b>T</b><b>V</b><b>N</b><b>D</b><b>V</b>  <b>K</b>A<b>N</b><b>G</b><b>K</b><b>T</b><b>P</b><b>N</b><b>D</b><b>Y</b>  ... </p>	<p> <b>E</b>...<b>F</b>K<b>E</b><b>C</b><b>C</b><b>S</b><b>I</b><b>T</b>  <b>G</b>I<b>R</b><b>L</b><b>S</b><b>E</b><b>S</b><b>G</b><b>E</b>  ... </p>	<p> 1235 1091 1294 </p>
Rpps N L6	<p> <b>I</b>K<b>E</b><b>C</b><b>G</b><b>V</b><b>R</b><b>L</b><b>L</b><b>Y</b>  <b>N</b><b>E</b><b>K</b><b>M</b><b>Y</b><b>G</b><b>L</b><b>R</b><b>L</b><b>L</b><b>Y</b>  ... </p>	<p> ...<b>K</b><b>E</b><b>G</b><b>P</b><b>E</b><b>V</b><b>N</b><b>A</b><b>L</b><b>L</b>  <b>K</b><b>E</b><b>G</b><b>P</b><b>E</b><b>V</b><b>N</b><b>A</b><b>L</b><b>L</b>  ... </p>	<p> ...<b>V</b><b>Y</b><b>Q</b><b>E</b><b>T</b>  <b>Q</b>M<b>R</b><b>E</b><b>N</b><b>S</b><b>N</b><b>E</b><b>P</b><b>T</b>  ... </p>	<p> <b>E</b>H<b>N</b><b>Q</b><b>Q</b><b>T</b><b>R</b><b>R</b><b>S</b><b>K</b>  <b>E</b>H<b>S</b><b>T</b><b>G</b><b>I</b><b>R</b><b>R</b><b>T</b><b>Q</b>  ... </p>	<p> <b>K</b>R<b>M</b><b>R</b><b>V</b><b>S</b><b>L</b><b>L</b><b>P</b>  <b>Y</b>N<b>N</b><b>R</b><b>T</b><b>S</b><b>F</b><b>Y</b><b>E</b><b>L</b>  ... </p>	<p> ...<b>I</b><b>N</b><b>G</b><b>L</b><b>I</b><b>A</b><b>A</b>  ...<b>I</b><b>N</b><b>G</b><b>L</b><b>I</b><b>A</b><b>A</b>  ... </p>	<p> 1269 1144 1294 </p>

Figure 4

ATGGCGGCTT	CTTCTTCTTC	TGGCAGACGG	AGATACGACG	TTTTTCCAAG
CTTCAGTGGG	GTTGATGTTT	GCAAGACGTT	CCTCAGCCAT	CTTCTCAAGG
CTCTCGACGG	CAAATCAATC	AATACATTCA	TCGATCATGG	AATCGAGAGA
AGCCGCACAA	TCGCCCCTGA	GCTTATATCG	GCGATTAGAG	AAGCTAGGAT
CTCAATCGTC	ATCTTCTCTA	AGAACTATGC	TTCTTCAACG	TGGTGCTTAA
ATGAATTGGT	TGAGATCCAC	AAGTGCTTTA	ATGATTTAGG	TCAAATGGTG
ATTCCAGTTT	TCTACGACGT	TGATCCTTCG	GAAGTTAGAA	AACAGACCGG
CGAATTTGGA	AAGGTCTTTG	AAAAGACATG	CGAGGTCAGC	AAGGACAAAC
AACCAGGGGA	TCAGAAACAA	AGATGGGTGC	AAGCTCTCAC	AGATATAGCA
AATATAGCCG	GAGAGGATCT	TCTGAACGGG	CCTAATGAAG	CGCATATGGT
TGAAAAGATA	TCCAATGATG	TTTCGAATAA	ACTTATCACT	CGGTCAAAGT
GTTTTGATGA	CTTCGTCGGA	ATTGAAGCTC	ATATTGAGGC	AATAAAATCA
GTATTGTGCT	TGGAATCCAA	GGAAGCTAGA	ATGGTCGGGA	TTTGGGGAACA
GTCAGGGATT	GGTAAGAGTA	CCATCGGAAG	AGCTCTTTTC	AGTCAACTCT
CTAGCCAGTT	CCACCATCGC	GCTTTCCTAA	CTTATAAAAG	CACCAGTGGT
AGTGACGTCT	CTGGCATGAA	GTTGAGTTGG	CAAAAAGAGC	TTCTCTCGGA
AATCTTAGGT	CAAAAGGACA	TAAAGATAGA	GCATTTTGGT	GTGGTGGAGC
AAAGGTAA	TCACAAGAAA	GTTCTTATCC	TTCTTGATGA	TGTGGATAAT
CTAGAGTTTC	TTAAGACCTT	GGTGGGAAAA	GCTGAATGGT	TTGGATCTGG
AAGCAGAATA	ATTGTGATCA	CTCAAGATAG	GCAACTTCTC	AAGGCTCATG
AGATTGACCT	TGTATATGAG	GTGAAGCTGC	CATCTCAAGG	TCTTGCTCTT
AAGATGATAT	CCCAATATGC	TTTTGGGAAA	GACTCTCCAC	CTGATGATTT
TAAGGAATA	GCATTTGAAG	TTGCCGAGCT	TGTCGGTAGT	CTTCCTTTGG
GTCTCAGTGT	CTTGGGTTC	TCTTTAAAAG	GAAGGGACAA	AGATGAGTGG
GTGAAGATGA	TGCCTAGGCT	TCGAAATGAT	TCAGATGATA	AAATTGAGGA
AACACTAAGA	GTCGGCTACG	ATAGGTTAAA	TAAAAAAAAT	AGAGAGTTAT
TTAAGTGCAT	TGCATGTTTT	TTCAATGGTT	TTAAAGTCAG	TAACGTCAAA
GAATTACTTG	AAGATGATGT	TGGGCTTACA	ATGTTGGCTG	AGAAGTCCCT
CATACGTATT	ACACCGGGTG	GATATATAGA	GATGCACAAT	TTGCTAGAGA
AATTGGGTAG	AGAAATTGAT	CGTGCAAAGT	CCAAGGGTAA	TCCTGGAAAA
CGTCAATTTT	TGACGAATTT	TGAGGATATT	CGAGAAGTAT	TGACCGAGAA
AACTGGGACC	GAAACTCTTC	TTGGAATACG	TTTGCCACAC	CCGGGATATC
TTACGACAAG	GTCGTTCTTA	ATAGATGAAA	AATCATTCAA	AGGCATGCGT
AATCTCCAAT	ATCTAGAAAT	TGGTTATTGG	TCAGATGGGG	TTCTACCTCA
GAGCCTCGTT	TATTTCCCTC	GTAAACTCAA	AAGGCTATGG	TGGGATAATT
GTCCATTGAA	GCGTTTGCCT	TCTAATTTTA	AGGCTGAGTA	TCTGGTTGAA
CTCAGAATGG	TGAATAGTAA	GCTTGAGAAG	CTGTGGGATG	GAACCTCAGCC
CCTTGGAAGT	CTCAAGAAGA	TGGATTTGTA	TAATTCCTAC	AAATTGAAAG
AAATTCCAGA	TCTTTCTTTA	GCCATAAACC	TCGAGGAATT	AAATCTTGAA
GAATGCGAAT	CTTTGGAGAC	ACTTCCTTCC	TCGATTGAGA	ATGCCATTAA
ACTGAGGGAG	TTAAATTGTT	GGGGGGGGCT	ATTAATAGAT	TTAAAATCAT
TAGAAGGCAT	GTGTAATCTC	GAATATCTAT	CAGTTCCTAG	TTGGTCAAGT
AGGGAATGCA	CTCAGGGCAT	CGTTTATTTT	CCTCGTAAAC	TCAAAAGTGT
ATTGTGGACT	AATTGTCCAT	TGAAGCGTTT	GCCTTCTAAT	TTTAAGGCTG
AGTATCTGGT	TGAACCTATA	ATGGAGTACA	GTGAGCTTGA	GAAGCTGTGG
GATGGTACTC	AGTCACTTGG	AAGTCTCAAG	GAGATGAATT	TGAGGTATTC
CAACAATTTA	AAAGAAATTC	CAGATCTTTC	TTTAGCCATA	AACCTCGAGG
AATTAGATCT	TTTTGGATGC	GTATCTTTGG	TGACACTTCC	TTCTCGATT
CAGAATGCCA	CTAAACTGAT	CTATTTAGAT	ATGAGTGAAT	GCGAAAATCT
AGAGAGTTTT	CCAACCGTTT	TCAACTTGAA	ATCTCTCGAG	TACCTCGATC

TCACTGGATG CCCGAATTTG AGAAATTTCC CAGCAATCAA AATGGGATGT  
GCCTGGACTA GATTATCTCG AACAAAGATTG TTTCCGGAAG GGAGAAATGA  
GATCGTGGTA GAAGATTGTT TCTGGAACAA GAATCTCCCT GCTGGACTAG  
ATTATCTCGA CTGCCTTATG AGATGTATGC CTTGTGAATT TCGCTCAGAA  
CAACTCACTT TTCTCAATGT GAGCGGCTGC AAGCTTGAGA AGCTATGGGA  
AGGCATCCAG TCGCTTGGAA GTCTCGAAGA GATGGATCTG TCAGAATCTG  
AAAACCTGAA AGAACTTCCA GATCTTTCAA AGGCCACCAA TCTGAAGCTT  
TTATGTCTCA GCGGGTGCAA AAGTTTGGTG ACACTTCCTT CTACAATTGG  
GAATCTTCAA AATTTGAGAC GTTTGTACAT GAACAGATGC ACAGGGCTGG  
AGGTTCTTCC GACCGATGTC AACTTGTTCAT CTCTCGAAAC CCTCGATCTC  
AGTGGTTGCT CAAGTTTGAG AACTTTTCCT CTGATTTCAA CTAATATTGT  
ATGTCTCTAT CTGGAAAACA CCGCCATTGA AGAAATTCCA GATCTTTCAA  
AGGCCACCAA GCTCGAGTCT TTGATACTCA ACAACTGCAA AAGTTTGGTG  
ACACTTCCTT CTACAATTGG GAATCTTCAA AATTTGAGAC GTTTGTACAT  
GAACAGATGC ACAGGGCTGG AGCTTCTTCC GACCGATGTC AACTTGTTCAT  
CTCTCGAAAC CCTCGATCTC AGTGGTTGCT CAAGTTTGAG AACTTTTCCT  
CTGATTTCAA CTAGAATCGA ATGTCTCTAT CTAGAAAACA CCGCCATTGA  
AGAAGTTCCC TGCTGCATTG AGGATTTTCC GAGGCTCACT GTACTACGGA  
TGTATTGTTG CCAGAGGTTG AAAAACATCT CCCCAAACAT TTTCAGACTG  
ACTAGTCTTA CGCTCGCCGA CTTTACAGAC TGTAGAGGTG TCATCAAGGC  
GTTGAGTGAT GCAACTGTGG TAGCGACAAT GGAAGATCCG ATTTCTTGTTG  
TACCATTATC TGAAAACATT GAATATACAT GTGAACGTTT CTGGGATGCG  
TGTTCCTGATT ATTACTCTGA TGACTTTGAG GTAAATCGGA ACCCAATTAG  
ATTGTCAACG ATGACTGTCA ACGATGTGGA GTTTAAGTTT TGTTGCTCCA  
TTACGATCAA AGAATGCGGT GTACGACTCT TGTATGTCTA TCAAGAAACA  
GAGCACAACC AACAACTAC GAGAAGCAAG AAGCGGATGC GGGTAAGCCT  
TTTGCCA

## INTERNATIONAL SEARCH REPORT

Internat Application No  
PCT/GB 96/00849

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C12N15/29 C12N15/82 A01N65/00 C12Q1/68 A01H5/00

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C12N A01N C12Q A01H

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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☒ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

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Date of the actual completion of the international search

28 August 1996

Date of mailing of the international search report

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Maddox, A



## INTERNATIONAL SEARCH REPORT

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## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

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Internat. Application No

PCT/G8 96/00849

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		WO-A-	9528478	26-10-95
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WO-A-9505731	02-03-95	NONE		
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